

lesions (BML), osteophytes, and cartilage lesions (CL)) separately. We used multivariate logistic regression models with generalized estimated equations to calculate odds ratios (95% confidence intervals). To investigate possible effect modification we additionally stratified by obesity (body mass index ≥ 27 kg/m²) and menopausal status.

Results: Within the study population, 4.6% of the knees had KL ≥ 2 , 9% of the knees had TFOAMRI, 5.8% fulfilled the criteria for medial TFOAMRI, 2% the lateral TFOAMRI, 7.7% showed PFOAMRI, 27% BML, 15% Osteophytes, and 17% CL. Carotid intima-media-thickness (mean IMT = 0.9mm; sd:0.1) and the presence of a carotid plaque (62%) showed no associations with knee OA (medial, lateral, or total TFOAMRI or K&L ≥ 2), nor with any of the MRI or X-ray-features of early knee OA. Stratification by body mass index or menopause did not change our results.

Conclusions: In this cross-sectional study, no associations between carotid intima-media thickness or carotid plaque and early signs of knee OA were found among middle-aged women. With these vascular measurements and these early knee OA features we could not find an association as we found earlier in the older women (mean age 68.6 years). This might be due to the low prevalence of KOA in these middle-aged women or there is no relationship in this younger female population (mean age 55.0 years).

408

LOCALLY INDUCED EXPERIMENTAL OSTEOARTHRITIS RESULTS IN A SYSTEM BONE MARROW SHIFT TOWARDS A PRO-INFLAMMATORY MONOCYTE SUBPOPULATION

E.J. Geven, A. Sloetjes, I. DiCeglie, W. de Munter, S. van Dalen, G. Ascone, P.L. van Lent. *Radboud Univ. Med. Center, Nijmegen, Netherlands*

Purpose: A significant role for inflammation during osteoarthritis (OA) is increasingly recognized, which involves the recruitment of immune cells, including monocytes, towards the inflamed synovium. In mice two functionally distinct monocyte populations are described; Ly6C-high monocytes, which express CCR2 and are considered pro-inflammatory and Ly6C-low monocytes, which express CX3CR1 and are suggested to be involved in repair processes. These monocytes arise from the bone marrow (BM) where monocyte chemoattractant protein-1 (MCP-1) is a key molecule that drives the monocyte efflux. As second tissue from which monocytes may originate is the spleen. The aim of this study is to investigate systemic effects of locally induced OA on BM and splenic monocyte subpopulations and the recruitment of these monocytes to the OA joint synovium in collagenase induced osteoarthritis (CiOA).

Methods: CiOA was locally induced in C57Bl/6 mice by injection of collagenase in the right knee joint. Seven and 42 days after induction, mice (n=6) were sacrificed, together with age-matched naive C57Bl/6 mice. Cells from BM, spleen, blood and knee synovial tissue were isolated and analyzed by FACS. Ly6C-high monocytes were identified as (B220/CD90/CD49b/NK1.1/Ly6G)-low CD11b-high (F4/80/MHCII/CD11c)-low Ly6C-high and Ly6C-low monocytes as (B220/CD90/CD49b/NK1.1/Ly6G)-low CD11b-high (F4/80/MHCII/CD11c)-low Ly6C-low. BM expression of MCP-1, CCR2 and CX3CR1 mRNA was determined at day 7 and 42 by q-PCR.

Results: In naive synovium few monocytes were present (1012 ± 925 Ly6C-high and 621 ± 488 Ly6C-low monocytes), which is likely an artefact of residual blood. At day 7 after CiOA induction, the number of Ly6C-high and -low monocytes in the OA synovium was 420% and 300% increased, respectively, compared to naive synovium. In blood, monocyte subpopulations were not changed, but in BM, the number of Ly6C-high monocytes was 160% increased, while Ly6C-low monocytes were decreased by 170%. Furthermore, expression of MCP-1 and CCR2 was increased by 3.2 and 2.8 times, while CX3CR1 expression remained unchanged. Even at day 42 increased levels of both monocyte subpopulations were observed in the OA synovium (Ly6C-high; 280% and Ly6C-low; 220%). In the BM, no change in both monocyte subpopulations was observed anymore as well as no change in expression of MCP-1, CCR2 and CX3CR. In spleen no changes in Ly6C-high or -low monocytes were observed throughout the course of CiOA, indicating no role for splenic monocytes in the recruitment of monocytes towards the OA synovium.

Conclusions: These data indicate that compared to naive synovium, increased numbers of both Ly6C-high and -low monocytes are present in the OA synovium throughout the course of CiOA, but that a systemic effect on the BM monocyte subpopulations and their efflux is only

observed in the early phase of OA. In the BM a clear skew towards a pro-inflammatory monocyte subset is visible, indicating that locally induced OA may also be systemically regulated.

409

ATTENUATION OF HYALURONAN FRAGMENT INDUCED INFLAMMATORY RESPONSE IN MACROPHAGES BY CHONDROITIN SULPHATE

T. Stabler[†], E. Montell[‡], J. Verges[‡], V.B. Kraus[†]. [†]Duke Molecular Physiology Inst., Durham, NC, USA; [‡]Bioiberica, Barcelona, Spain

Purpose: Hyaluronan (HA) fragments (<500 kDa) are known to be pro-inflammatory and able to induce an inflammatory response from macrophages characterized by the release of IL-1 β and other pro-inflammatory cytokines. We have previously shown that chondroitin sulphate (CS) can attenuate the monosodium urate crystal mediated release of inflammatory cytokines from activated macrophages in culture. We sought to determine whether CS could similarly ameliorate an HA fragment mediated inflammatory response and whether any CS inflammatory inhibition was acting at the level of the inflammasome.

Methods: Cell culture: The THP-1 human monocytic cell line (ATCC TIB-202) was grown in RPMI 1640 with HEPES and supplemented with glucose, pyruvate, 2-mercaptoethanol, 10% FBS and penicillin/streptomycin as recommended by ATCC. These cells were grown to a density of 1.5×10^6 cells/ml, plated onto a 96 well plate at a density of 2×10^4 cells/well, then induced to differentiate into mature macrophages by the addition of 200 nM of a phorbol ester (12-O-tetradecanoylphorbol-13-acetate). After 2 days the media was replaced with media without the phorbol ester and grown for an additional 2 days. Cells were primed with a very small amount (10 ng/ml) of LPS for 24 hours with CS in various physiologically relevant concentrations (0, 10–200 μ g/ml). After 24 hours, cells were washed with PBS and media replaced with serum free Opti-MEM along with the previously mentioned concentrations of CS and various molecular weights (sizes) (ULMW=7.5 kDa, LMW=29 kDa, MMW=289 kDa, and HMW= 1.54×10^6 kDa) and concentrations (0, 1, 10, and 100 μ g/ml) of HA fragments were added to the media. After a further 24 hours, cell viability was assessed and the media harvested. Following a PBS wash, water was added to the remaining cells and plates were subjected to 3 sonication/freeze/thaw cycles in order to release the cell contents. Media and cell contents were snap frozen until further analysis. Caspase-1 experiments were carried out in 25cm² flasks and activity was measured on fresh lysate derived from 2×10^6 cells.

Biochemical measurements: Media and cell contents were analyzed for IL-1 β and proIL-1 β by ELISA (R&D Systems). Caspase-1 activity was determined by fluorometric assay (R&D Systems). Cell viability was measured using PrestoBlue reagent (Invitrogen).

Statistical Analysis: After normalization for cell viability, all results were expressed as fold change from the media only negative control (no CS or HA). One-way ANOVA with Dunnett's post-hoc test and post-hoc linear trend were performed using Graphpad Prism software.

Results: As expected, HA fragments produced large increases ($p < 0.0001$) in IL-1 β release at 10 and 100 μ g/ml, with a decreasing gradient effect ($p < 0.0001$) seen from ULMW to MMW and no effect seen for HMW HA. CS (100–200 μ g/ml) produced a dose dependent reduction in IL-1 β release in cells treated with 10 μ g/ml of the 3 HA lower MW fragment types (fig 1). While ULMW HA fragments induced a significant increase ($p < 0.0001$) in intracellular caspase-1 activity, CS had no effect on this activity. CS reduced intracellular IL-1 β and proIL-1 β in cells treated with ULMW HA, however, the ratio between the 2 was unchanged (fig 2).

